THE PATHOLOGIC CHANGES CAUSED BY CERTAIN SO-CALLED TOXALBUMINS.

An Experimental Study.

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An Experimental Study.

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THE crystalline principles called ptomaïns which have been isolated by Selmi, Nencki, Brieger,[2] and others from cultures of bacteria do not, as was once supposed, represent the essentially active agents produced by pathogenic bacteria. Since the isolation by Roux and Yersin[3] in 1888, from cultures of the bacillus diphtheriæ, of an amorphous toxic albuminous substance which, as they showed, was capable of causing all of the symptoms of diphtheria, excepting the pseudo-membrane, other albuminous substances have been obtained from a number of pathogenic bacteria.

The researches of Kobert and Stillmark,[4] Martin,[5] and Hellin[6] have led to the isolation from the castor-bean and the jequirity-bean of two amorphous substances—ricin and abrin respectively—which in many

[1] Read before the Philadelphia Pathological Society, April 26, 1894.

[2] Berliner klin. Wochenschrift, 1889, No. 39.

[3] Annales de l'Inst. Pasteur, 1888, No. 12, p. 629

[4] Kobert, Lehrbuch d. Intoxicationen, 1893; also, Kobert and Stillmark, Arbeiten des pathologischen Institutes, Dorpat, 1889, iii.

[5] Martin: Proceedings Royal Soc., London, vol. xlvi.

[6] Hellin: Dissertation, Dorpat, 1891.

respects resemble the toxic albuminous principles obtained from bacteria.

We owe to the investigations of Weir Mitchell and Reichert[1] the knowledge that the toxicity of snake-venom depends upon certain albuminous constituents, and later Mosso and Springfeld[2] separated from the blood of eels an exquisitely poisonous albuminous substance. Moreover, the well-known effects which the blood-serum of one species of animal exerts upon another species has led to the conclusion[3] that the blood of different animals contains distinctly toxic substances.

As to the precise nature of many of these substances, to which the name of toxalbumins has been provisionally given, there is little agreement. They are regarded by some investigators as being enzymes, while a few deny altogether their albuminous nature.

It has been suggested by Roux and Yersin that the diphtheria-toxalbumin is an enzyme, and Stillmark holds the same view concerning ricin, while Nencki[4] considers that the proteids of the blood-serum, to which it is believed by many that animals owe their immunity from disease, belong to the same category.

The recent statement of Brieger and Cohn,[5] based upon the study of the properties of a highly refined tetanus-toxin, that this body, which fails to react with Millon's reagent, and responds to the xantho-proteic test, is not an albumin, is not regarded as sufficient to disprove its proteid nature.[6]

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[1] Smithsonian Contributions, Washington, 1886.

[2] Rend. Accad. Linc., Rome, 1888, 1889.

[3] Loew: Ein natürliches System der Giftwirkung, München, 1893, p. 79.

[4] Correspondenzblatt für Schweizer Aerzte, 1890.

[5] Zeitschrift für Hygiene, vol. xv, p. 1.

[6] Uschinsky (Centralblatt f. Bakteriologie, Band ix, p. 316) succeeded in cultivating the tetanus-organism in a medium composed of salts and asparagin, and free from albumin. The teta-

Brieger and Fränkel[1] oppose the view of the enzyme-nature of these bodies; and Fermi,[2] who also objects to this view, considers as militating against it the fact that of 114 described pathogenic microörganisms which produce toxins, only 26 produce proteolytic ferments, and that, on the other hand, of 134 known enzyme-producing bacteria only 25 are pathogenic—that is to say, toxalbumin-producing.[3]

The blood-serum of the dog acts with great energy upon the red blood-corpuscles of the rabbit, and Buchner[4] observed that while the blood-serum of these animals is separately capable of destroying a considerable number of typhoid-bacilli, yet when the serum of the dog and rabbit are mixed, the destructive influence of the mixture upon typhoid-bacilli is less than that possessed by either serum alone. As bearing on this question, the experiments of Robert Hartig[5] on the fungus-parasites of plants are interesting. Thus, while certain of the ferments of this origin dissolve the cellulose of plants and not the intercalated wood-gum, there are others that dissolve the gum and have no effect upon the cellulose. According to Hartig the oak is attacked by ten distinct parasites, each of which produces a different ferment. Two of these dissolve starch—are amylolytic—when acting separately, but when combined they are entirely without action on this substance. Does this not suggest an analogy with the mutual destruction or neu-

nus-poison obtained from this source still gave the reactions mentioned.

[1] Berliner klin. Wochenschrift, 1890, p. 241.

[2] Archiv für Hygiene, vols. ix and xiv.

[3] Fermi and Pernossi (Zeitschrift f. Hygiene und Infektionskrankheiten, Band xvi, Heft 3, p. 435) believe that they have proved that the enzyme produced by the tetanus-bacillus is distinct from the poison tetanin, and that the latter is not a ferment.

[4] Münchner med. Wochenschrift, 1892.

[5] Lehrbuch der Baumkrankeiten, Berlin, 1882. (Quoted by Loew, ibid.)

tralization of the bactericidal principles of the blood of the rabbit and the dog?

Further, we have grounds for believing that the blood possesses the power of destroying the true ferments, as it does certain of the bacterial products, and, moreover, that the true ferments may act in a manner similar to the vegetable toxalbumins in conferring immunity. Hammersten[1] has shown that if rennet and milk are injected separately into the circulation of rabbits the coagulating influence of the ferment upon the milk is much diminished; and Hildebrand[2] has demonstrated an antiferment in the blood of animals rendered immune from certain of the enzymes, especially emulsin. He[3] has succeeded, moreover, in rendering rabbits more resistant to the bacillus cuniculicida, and even of protecting them against the organism by previously inoculating them with and making them immune to emulsin.

Heating solutions of many of the toxalbumins or blood-serum to 60° C. destroys their activity. Blood-serum kept for thirty minutes at a temperature of 55° C. loses, according to Buchner, its power to destroy bacteria and likewise its globulicidal action. Exposed to a temperature of 54° C. for the same time I have found that dog's serum is still globulicidal for rabbits, although its activity is diminished.

Blood-serum that has lost its bactericidal and globulicidal properties by being kept at a temperature of 60° C. for thirty minutes has not suffered coagulation, nor have demonstrable optic or chemic changes occurred in it. The lost bactericidal property, it is believed by Emmerich and Tsuboi,[4] can be restored by alkalinization and dialysis, a circumstance that they bring forward as bearing upon

[1] Schmidt's Jahrbücher, 1887, Band ccxvi, p. 115.

[2] Virchow's Archiv, vols. cxxi, cxxii, cxxxi.

[3] Hildebrand: Münchener med. Wochenschrift, 1894, No. 15, p. 283.

[4] Centralblatt für Bakteriologie, vols. xii and xiii.

the question of the chemic *versus* the vital action of the serum. Buchner,[1] it must be mentioned, disputes the results of Emmerich and Tsuboi and claims that the difference that they observed in the effects of the heated serum before and after alkalinization is explicable on other grounds, as, for example, the alteration in the heated serum as a culture-medium. None of these authors, however, mentions the effect of this treatment upon the globulicidal property of the serum, and to determine this I have made the following experiments:

The blood-serum[2] of a dog, collected after twenty-four hours, was tested as to its toxicity, by injecting 1.5 per cent. of the body-weight into the ear-veins of a rabbit. The animal was profoundly affected, soon manifested hemoglobinuria, and died in ten hours.

A second half-grown rabbit, whose blood-count at 11.15 A.M. was 6,080,000 red corpuscles to the c.mm., received 1 per cent. of its weight of unheated serum. It was dead in five minutes, from thrombosis of the right side of the heart. A second-blood-count, made from the lake-colored blood of the left ventricle taken during the death-struggle, showed 3,872,000 red corpuscles per c.mm.

A third control-rabbit received 1 per cent. of its weight of the same serum heated to 55° C. for one hour, without any effect whatever.

Seventy-five c.cm. of the heated serum were now made alkaline with 7.5 c.cm. of a 3 per cent. solution of pure caustic soda and dialyzed through gut for forty-three hours against seventy-six liters of normal salt-solution. The whole volume of serum now equalled 90 c.cm.

A white-mixed rabbit weighing 1600 grams, whose blood-count on March 21st was 5,664,000, and on March 22d 6,368,000 per c.mm., received at 10.20 A.M. an amount of dialyzed serum which corresponded to 1 per cent. of the body-weight of the original serum (16 c.cm. of the original = 19.5 c.c. of dialyzed serum). No immediate effect was produced. At 11.10 A.M. the blood-

[1] Centralblatt für Bakteriologie, vol. xii, p. 855.

[2] In all cases in which blood-serum was used the drawn blood was placed upon ice immediately after having been obtained.

count was 4,938,000 per c.mm. No hemoglobinuria or other unfavorable symptom had appeared. On April 11th the rabbit was still alive and apparently well, the blood-count on this day showing 5,856,000 per c.mm.

Another rabbit, weighing 1800 grams, whose blood-count on March 21st was 6,112,000 per c.mm., and on March 22d 6,208,000, received at 10.30 A.M., March 22d, 1.5 per cent of its body-weight (27 c.c. of the original = 32.5 c.c. of dialyzed serum) of the dialyzed serum. At 11.25 the blood-count was 5,180,000 per c.mm. At 2 P.M. this rabbit, which appeared perfectly well, received a second quantity of 1.5 per cent. of its body-weight. The blood-count at 3 P.M. showed 4,980,000, and on April 11th, at which time the animal appeared normal, it was 5,952,000.

The second animal, it will be noted, received double the certainly lethal dose of the normal, unheated serum, with relatively slight effect. From these experiments it must be concluded that the globulicidal action of the serum is certainly not entirely restored, and if at all, to a minor degree only, by alkalinization. The animals are still living and so far show no evidence of the toxic effects of the serum. Hence, it would appear that the toxic properties are also lost at this temperature.

In considering the pathologic action of the toxic proteids derived from the several sources mentioned upon susceptible animals we shall find reason to treat of them together. If we consider for a moment the production of immunity, which, for many bacteria and their products, is such a well-known fact that it need not detain us, we shall find that evidence is not wanting for the albumins derived from the higher plants, the phytalbumoses. However, up to the present time no one has succeeded in rendering animals permanently immune to the animal toxic proteids, although Sewell[1] claims to have secured in pigeons an immunity to snake-poison which disappeared after a time.

[1] Journal of Physiology, 1887.

Ehrlich[1] has shown that mice, which are relatively quite susceptible to ricin and abrin, can be rendered in a high degree immune from the action of these bodies. As concerns ricin, he found that a solution having a concentration of 1 to 750,000, in the quantity of 1 c.c. per 20 grams of weight will often kill mice in from two to four days; and in the same dose a solution of 1 to 200,000 will kill these animals without exception. The local effect of the drug is so intense that it is necessary to resort first to feeding, by which means a low grade of immunity is established, which can then be increased by subcutaneous inoculations. The immunity appears quickly. I have repeated Ehrlich's experiments and have confirmed his results.

For example, two mice were fed with gradually increasing doses for ten days, and at the end of this time an immunity equal to the natural resistance of five, as determined by subcutaneous inoculations with control-animals, was already present. From now on the doses were rapidly increased, so that on the twelfth day the resistance was equal to ten; on the sixteenth, to eighty; on the eighteenth, to one hundred and forty, and on the twentieth day, to two hundred and fifty.

These figures are all low, as the calculation is based on the surely lethal dose of 1 to 250,000, and the highest grade of immunity was not determined. Even at this time the immune animals were found not to be indifferent to the mode of introduction; for of two mice, which up to this point had acted in all respects alike, one was inoculated into the peritoneal cavity, and died in one and a half hours, while the other, after subcutaneous inoculation with the same amount (1 c.c. of a 1 to 1000 solution), became slightly sick, but was perfectly well the next day. The blood of the mouse that succumbed was laky. The blood of immune animals, *i. e.*, in a condition that Ehrlich denominates ricin-proof, contains an

[1] Deutsche med. Wochenschrift, 1891.

antitoxic body, which by simple admixture outside the body with a solution of ricin is capable of rendering the latter innocuous.

Having in mind the production of immunity from this substance, I have experimented on rabbits with dog-serum, but so far without success. On the contrary, I found that animals that had withstood one dose of dog's serum would succumb to a second dose, given after the lapse of some days, or weeks, even when this dose was sub-lethal for a control-animal.

Two rabbits received ½ of 1 per cent. and 1 per cent. of their body-weight respectively of dog's serum, twenty-four hours old, on January 19, 1894. With the exception of hemoglobinuria, indisposition to move, and increased respiration, no ill-effects were noted. The animals still showed hemoglobinuria on the following day. These symptoms disappeared, and apparently the rabbits entirely recovered. On February 12, 1894, each received 1 per cent. of their body-weight of dog's serum intravenously. A control-animal also received 1 per cent. of its body-weight of the same serum. The two animals that had been previously inoculated died in two and twelve hours respectively; the control-animal showed only hemoglobinuria, which disappeared after a day or two.

The pathologic lesions produced in animals by these various poisonous substances have been very imperfectly studied up to the present time. The contributions that have been made to this subject refer almost exclusively to the gross changes in organs and tissues, or consider simply the effect that is produced upon the blood, especially the alterations in coagulability. Hellin, who studied, he says, one hundred organs or parts of organs obtained from animals dead of abrin-poisoning, could discover nothing abnormal, except thrombi composed of red blood-corpuscles. The whole pathologic process in these cases has been considered to depend upon an active gastro-enteritis, thrombosis of the vessels of the

stomach and intestine, necrosis and ulceration from digestion. To this is to be added, for those cases in which death occurs rapidly with symptoms of convulsions, thrombosis of the cerebral vessels. Just as little attention has been given to the tissue-changes caused by the inoculation of animals with foreign serum. Heretofore it has sufficed to consider as the cause of death in the immediately fatal cases the coagulation of the blood, especially in the right heart and main pulmonary vessels, or the production of widespread capillary thrombosis. All experimenters have, however, encountered cases in which, as death has been delayed for several days, this explanation was insufficient, as thrombi were not demonstrable. Ponfick has shown that the kidneys suffer injury in secreting the hemoglobin liberated by the breaking up of the red blood-corpuscles, and he attributes many cases of death to the resulting nephritis and blocking of the kidney-tubules with methemoglobin casts.

It will be my especial purpose to point out the insufficiency of these views. A study of the pathologic changes in the organs has convinced me that the extent, and not the absence of tissue-alterations, is most remarkable.

At this time there are comparatively few that accept the bacterial origin of disease who will question the mode in which the organisms act, *i. e.*, whether it is the presence of the bacteria as such in the tissues, or the products of their vital activities, that is responsible for the harm that is done. Neither the mechanical influence, nor the effects of depriving the body of a certain quantity of oxygen and albumin, will longer suffice to explain their action. It is true that as living beings the bacteria require certain materials in order to build up their bodies and maintain their functions, and these they secure from the available stores of their surroundings, but it is just as certain that in the performance of their activities they produce excrementitious substances,

which are in turn discharged into the host. It is to these substances, of which we have already spoken, that the attention of chemists and pathologists has of late been directed. And the study of their composition and properties has not only brought much light into this field of work, but has opened up new avenues of investigation, at the same time awakening new and unexpected hopes for the future of therapeutics.

It is now some three years since Professor Welch and myself[1] published a short communication on the lesions produced in the tissues by the soluble products of diphtheria-bacilli. This paper, following one describing the histologic lesions in kittens, guinea-pigs, and rabbits caused by the inoculation of the bacilli themselves, confirmed in all essential respects the first paper.

The study of the relation of the bacillus diphtheriæ to the disease diphtheria indicated that the constitutional symptoms of the disease, which, in human beings, are often of the gravest nature, were due to an absorbed poison, and not to the organisms themselves. The separation of the poison from cultures in a dry, but impure state, by Roux and Yersin, and the reproduction through its agency of the constitutional symptoms, including the paralysis, of the disease may be said to have been conclusive. Yet it remained to find in the animals experimented upon lesions similar to or identical with those described by Oertel in human beings.

I shall not here undertake to give an account in detail of these changes. They have since been seen and studied by a number of investigators, and constitute an additional link in the chain of evidence of the causal relation of the bacillus diphtheriæ to diphtheria in human beings. But I wish to state that these lesions are found extensively in the tissues. They are present in the lymphatic glands generally, in the spleen, liver, intestinal canal, kidney, and heart-muscle, and are characterized by a

[1] Johns Hopkins Bulletin, 1891.

death of cells in the affected parts. Strangely enough, the peculiar changes are not equally distributed throughout the affected organs, as one would naturally infer when it is considered that we have to deal with a soluble substance circulating in the blood; but in the lymph-glands and viscera these lesions tend to occur in well-marked areas or foci.

This, then, has brought us face to face with a new problem in pathologic histology. Heretofore we were wont to separate the focal from the diffuse lesions. The latter class, such as commonly attend infections and intoxications, we have long since learned to know under the term given it by Virchow, of "parenchymatous degeneration," the "cloudy swelling" of the older writers. This is the type of lesion that was believed to result from the action of a soluble poisonous substance, in a certain concentration when the influence is continued for some time. The focal lesions, such as tubercle, a focal abscess, were regarded as the result of the lodgment and increase of the particular injurious agent in the areas affected.

Nor are we required to alter our views of the relation of the focal lesions in diphtheria to the soluble poison by the observation of Frosch,[1] since confirmed by several others, that in human beings a few diphtheria-bacilli are often found in the liver, the spleen, and other organs. The number of organisms is, in the first place, altogether insufficient to explain the lesions; there is, moreover, an entire absence of evidence of relation between the organisms and the tissue-changes, and, finally, there are cases in which no organism can be demonstrated, and, in animals, we now know that they are not necessary to the production of the focal lesions.

But I wish to present still more conclusive evidence of the relation between soluble poisons and focal lesions.

[1] Zeitschrift f. Hygiene u. Infektionskrankheiten, Band xiii, Heft 1, p. 49.

For example, certain lesions ought long ago to have led to the suspicion that even soluble substances may act upon one part of the body and not upon another.

Consider, for instance, the action of snake-poison. What could be more impressive than the production of capillary focal extravasations of blood in the serous membranes in consequence of a subcutaneous inoculation of the poison? In this case the action of bacteria or other essentially "living agent" can, by the experiments of Mitchell, Reichert, Formad, and others, be entirely excluded.

I have made a series of experiments with poisons of another kind. From the published accounts of the action of ricin and abrin upon animals, little can be learned concerning the minute pathologic changes induced by these bodies. Manifestly these substances offer an opportunity to study the question of the relation of soluble poisons to focal lesions. In them we have, as I have already shown, substances that in many respects resemble the bacterial poisons; and according to Martin, the action of abrin is suggestive of snake-poison. They thus seemed to constitute ideal substances for study.

The first experiments which I made showed me that the picture of the pathologic changes given by Kobert, Hellin, Stillmark, and Ehrlich was very incomplete. In the main, subsequent work enabled me to confirm their results; but they have overlooked, it seems to me, the most striking and significant changes.

The samples of ricin and abrin which I employed in my experiments were made by Merck. The substances, which are supplied in the form of grayish-white amorphous powders, are insoluble in water, but dissolve readily and completely in a 10 per cent. salt-solution. After solution they can be diluted without change by addition of physiologic salt-solution or distilled water.

One of the impressive results in the use of these bodies was the certainty of their action. After one has experi-

mented with bacteria, the virulence of which is open to fluctuation, this constancy of action is a very striking characteristic. In this respect, again, we can see an analogy with snake-poison. Kobert has pointed out that 0.03 mg. per kilogram of ricin is fatal to rabbits and cats, and probably dogs, if injected into the veins, and Ehrlich has shown that guinea-pigs are so susceptible that one gram of the substance would suffice to kill not less than 1,500,000 of these animals.

My experiments have been completed only so far as to enable me to report upon the effects produced upon mice, guinea-pigs, and rabbits. I have had among these animals very acute and chronic cases. The acute cases present such a characteristic clinical and pathologic picture as to be unmistakable.

The lesions about to be described could be produced almost with the certainty of a chemic reaction.

I shall not give the detailed protocols of the cases, but will content myself with a description of the pathologic changes in general, and shall, in the first place, summarize a few typical cases.

The intra-venous inoculation of ricin dissolved in 10 per cent. salt-solution in the proportion of 0.3 mg. to 3 mg. per kilogram of weight kills rabbits in from eighteen to twenty-six hours. In the cases of most rapid death, the axillary, inguinal, bronchial, and cervical lymphatic glands are found slightly swollen and softened. The peritoneal cavity may or may not contain an excess of clear fluid; the mesenteric glands at the attachment of the mesentery are also swollen and edematous. The intestines, especially the small intestine, are distended and pale, their contents being soft, glutinous, and grayish-white in color, and resembling in appearance cholera-stools. The patches of Peyer, in both the small and the large intestine, are elevated, swollen, and pale. They present a reticulated aspect in consequence of the greater swelling of the lymphatic follicles than of the interven-

ing mucous membrane. The heart is filled with blood, the right ventricle being especially distended. The blood is fluid; or soft, dark clots may appear. Neither the kidneys nor the lungs show especial alteration. The spleen is slightly swollen and softened, the liver large, soft, and yellow in color.

The animals that receive a smaller dose and live longer, say from twenty to twenty-six hours, show more pronounced lesions. All the superficial lymphatic glands are greatly swollen and dark in color. The spleen is often much enlarged, tense, and deep purplish-red in color. The kidneys are congested; sometimes the adrenals also. The mesenteric glands are much swollen, edematous and hemorrhagic. The peritoneal cavity often contains bloody fluid, and there are many subserous hemorrhages into the peritoneal layer of the intestines and mesentery. In some cases there are so many hemorrhages into the omentum that the picture presented reminds one of snake-poisoning. The intestines are distended with semi-fluid, glutinous contents, which often show streaks of blood. The mucous membrane of the intestines is congested, and here and there small hemorrhages are observed; the latter correspond at times with dilated bloodvessels in the serosa filled with dark, clotted blood. Peyer's patches present the most striking lesions, and are uniformly swollen and congested. The swelling is apparent at once through the serosa upon opening the peritoneal cavity and examining the intestines, and the glands often reach such a size that they project into the lumen of the intestine for a distance of two millimeters. At times small points of hemorrhage occur in and around the swollen patches, which always present a reticulated surface. In the stomach ulcers are sometimes found. The liver is dark in color, often much congested, and presents to the naked eye foci of yellowish or yellowish-white color, which at times are surrounded by hemorrhagic zones. A few of the inocu-

lated animals were pregnant, and in these there were without exception hemorrhages into the fetal sacs.

The microscopic changes are well marked. In the lymph-glands hemorrhages are commonly present, and the vessels are greatly dilated and packed with red blood-corpuscles. Many of the lymphatic elements are necrotic; cells with fragmented nuclei are common, and this fragmentation is often extensive. Intermingled with the foci of fragmentation are numerous karyokinetic figures. The lymphatic apparatus of the intestines shows in an analogous manner the destructive influence of the poison, but in this situation the cell-death is much more extensive, and the swelling of the follicles is clearly due in part to an increase in number of lymphoid elements. Nuclear figures are found in considerable numbers. In the intestines the destructive process is not limited to the lymphatic elements in the follicles, but those in the villi suffer extensively, and the epithelial elements are as little spared. Fragmentation and necrosis with rapid cell-multiplication by karyokinesis are present here also. The spleen shows changes similar to the lymph-glands, and these are localized in part in the Malpighian bodies.

The liver presents in many respects the most interesting changes, and a variety of forms of cell-death are met with in this organ. In the capillaries of the liver, which are greatly dilated, the endothelial cells are sometimes fragmented and necrotic, and the leukocytes suffer a similar change. But in the liver-cells themselves the process reaches its height. The yellowish and yellow-white areas visible to the naked eye correspond to foci of coagulation-necrosis of liver-cells. The liver-cells in these areas are still preserved in some instances, but they are hyaline in appearance and devoid of nuclei. At times the nuclei can still be made out in the form of fragments. In other foci the cells are much altered in appearance and staining properties, and the nuclei have undergone another form of necrosis. They have not

fragmented, but have died as a whole, becoming paler and paler until they finally fail to stain, with apparently a synchronous alteration in the cell-protoplasm. Still other cells have given up most of their protoplasmic material and appear as almost empty vesicles, with here and there a trace of protoplasm. Again, a group of cells will be converted into a reticulated substance suggesting fibrin; and, indeed, these foci of necrotic cells often give a reaction with Weigert's fibrin-stain, while the remainder of the tissue does not retain the dye.

These foci of necrosis are often surrounded by large quantities of nuclear detritus—to such an extent, indeed, as to exclude the possibility of its having been derived altogether from the tissue-nuclei. Some of the detritus must have come from emigrated cells, attracted, as we now know commonly happens, to these necrotic foci, where they suffer the same fate as the tissue-elements. An interesting feature was noticed in connection with the lymphatics in the interlobular spaces of the liver. They were at times choked with nuclear detritus. Manifestly such an amount of nuclear material could not come from the cells lining the lymphatics, nor from the cells of the lymph. There is only one interpretation possible of this appearance: it represents the nuclear detritus of the tissues and emigrated cells which had been swept by the lymph-vessels into the current. To accomplish this it is probable that an increased lymph-current is necessary.

That in the short space of time—about twenty-four hours—rapid cell-proliferation occurs in the lymph-glands we have adequate evidence, and we also have reason to believe that the same thing occurs in the liver. Around a focus of necrosis, about 1 mm. in diameter, in the periphery of which the capillaries were greatly dilated and literally choked with nuelear fragments, there were evidences of cellular proliferation in the rapid mul-

tiplication of the liver-cells, forming at times actual giant-cells.

The studies of Podwyssozki[1] have shown that in rabbits, after injury to the liver, cell-division by karyokinesis has already begun on the second day, and by that time the various stages of nuclear division are present. He also observed on one occasion liver-cells with colossal dividing nuclei, in which no indication of division of the cell-bodies could be made out; from these he believed that giant-cells were formed.

That the liver of rabbits under certain circumstances possesses marked capacity for regeneration has been shown by the experiments of Ponfick, who, after removing large portions of the organ, found that compensatory hypertrophy of the remaining parts took place.

In this description I have had nothing to say of the alterations of the blood described by Kobert. The thrombi of red blood-corpuscles which he found were also met with in my specimens. But they cannot, I think, be regarded as playing an essential part in the production of the lesions.

When we attempt to explain the phenomena that we have just described, great difficulties are at once encountered. There are, as will be readily admitted, several questions of no small interest involved. For example, what determines the localization of the activity of the poisonous agent to such a large extent upon certain structures? Why do the lymphatic elements everywhere suffer so disproportionately? Are there in the body special tissues whose function it is to destroy, render innocuous or eliminate the injurious substances? And as regards the lesions in the intestinal tract, is it not a matter of indifference whether we introduce the poisonous agent into the blood, by the stomach, or subcutaneously?

[1] Ziegler's Beiträge, vol. i.

Moreover how are we to account for the selection (for such it seems to be) of certain groups of cells on which the greatest stress of the poison is exerted? Shall we be obliged to fall back upon an hypothesis with which we are familiar enough in form, but as far as ever from understanding? I refer to the assumption of a difference of resistance of certain cell-groups of an organ, in consequence of which they fall an easy prey to the poisons which other cells can resist. Or, again, do the areas of necrosis, as has also been suggested, represent foci of especial enzyme-activity, points at which the poison increases, with the result finally of causing the death of the animal, and the lesion described?

These are fundamental questions and doubtless not all to be answered at the present time. But we have certain facts in our possession, derived from experiments, which may aid us in the solution of some of these problems.

Koeppe,[1] working in Ludwig's laboratory, and proceeding on the basis that the lymph flowing into a lymph-gland is poorer in cells than the outflowing lymph, supposed that if the lymph-entrance and lymph-exit were both closed an increase in the size of the gland would take place. He accordingly ligated the afferent and efferent lymph-vessels of one of the cervical lymphatic glands of the dog, and examined the gland, comparing it with its fellow of the other side. To his entire amazement, he found that after two weeks the gland operated upon was much smaller than its fellow, and showed upon microscopic examination fewer mitotic figures. The interpretation of this experiment seems clear: the lymph is the normal excitant of the lymph-gland, and upon its presence depends the production of lymph-corpuscles within the gland. This conclusion is made the more probable by the further observations of Koeppe, that the arterial blood supplied to the gland,

[1] His u. Braune's Archiv, 1891.

so far from being an aid, is a hindrance to the multiplication of the lymph-corpuscles.

From his experiments on lymph-formation, Heidenhain believed that he had proved that the separation of lymph from the blood does not depend upon a process of filtration, but is to be regarded as due to a secretory activity of the cells lining the capillaries. According to him a number of substances are capable of so acting on the capillary walls in the dog as to cause an elimination of an increased quantity of lymph, amounting often to several times the usual amount as measured by the outflow from the thoracic duct. Among the most active agents are the enzymes, derived from the salivary, pancreatic, and gastric secretions. Although Heidenhain[1] considered that his experiments proved that the increased lymph-flow is quite independent of the blood-pressure, yet Starling[2] has just shown that in a number of his experiments there was an increased capillary pressure in the liver, whence a large part of the lymph was derived, and it will be recalled that in the liver in our experimental animals the capillaries are much dilated, often compressing the rows of liver-cells.

These facts are, I think, very suggestive. On the one hand we have learned that the lymph is an excitant of the lymphatic glands, and on its presence depends the formation of lymphoid cells, and, on the other hand, it has been demonstrated that certain ferments may act as the cause of an increased lymph-flow. It is pertinent, I think, since the histologic evidence that an increased flow was actually induced by these enzyme-like bodies is before us, to inquire whether or not the rapid increase in the lymphatic elements can in part be attributed to this cause.

The variations in the circulation of the blood in the

[1] Pflüger's Archiv, Band xlix. See also Hamburger Zeitschrift für Biologie, Band xxx, p. 143.

[2] Starling: Arris and Gale Lectures on the Physiology of Lymph-formation, Lancet, 1894, 3683 et seq.

organs must be considered as bearing on the production of such focally distributed lesions as are here described. That the circulation is not equal in all parts of an organ at all times is indicated by observations upon living animals, and the capillary circulation especially is subject to wide variation.

I would ask a consideration of the effect this difference may have in bringing about such focal effects as we have encountered in the liver. We have seen that the action of the poisonous agent is exerted in part upon the capillary walls. The necrosis, fragmentation and regeneration of endothelium indicate this. I would also point out that the injury to the capillary wall is nowhere so great as in the areas in which the necrosis is found. This fact suggests the possibility that, in certain capillaries in which the circulation was much diminished in rapidity or was temporarily at a standstill at the time when the irritant acted with the greatest intensity, such damage was done the capillary wall that a freer transudation took place into the tissues than elsewhere, resulting in the destruction of cell-groups; and Cohnheim[1] has shown that the lymph will be more concentrated the greater the permeability of the vessel-wall.

I have already alluded to the fact that in such areas there is an abnormal accumulation of leukocytes. The general blood contains an increased number of these cells, but there are relatively many more in the areas of necrosis.

We now know, thanks to the researches of Stahl,[2] Pfeffer,[3] Leber,[4] Buchner,[5] and others,[6] that certain

[1] Allgemeine Pathologie, 1882.

[2] Stahl: Botanische Zeitung, 1884.

[3] W. Pfeffer: Untersuch. a. d. botan. Institut zu Tübingen, Bd. i and ii.

[4] Leber: Fortschritte der Medicin, 1888; Die Enstehung der Entzündung, etc., Leipzig, 1891.

[5] Buchner; Berliner klin. Wochenschrift, 1890.

[6] J. Massart and Bordet: Journal de la Soc. R. des Sciences Médicales et Naturelles de Bruxelles, 1890. Steinhaus: Die

stimuli of a chemic nature attract and repel these as well as other cells. In undergoing necrosis, certain chemic changes take place in the tissues, through which the leukocytes are attracted to them, but some bacterial products are still more positively chemotactic than are those substances derived from cells. In order that this attraction shall be exerted, Pfeffer discovered for the sperm-threads of ferns that the attracting substance could not be equally diffused throughout the fluid, but must exist in a greater concentration in one part than in others. There is then an immediate movement toward the areas of concentration. It seems plausible at least that the poison which is conceived to be in greater concentration in the areas of necrosis exerts a positively attractive influence upon the leukocytes. To test this point the following experiment was made.

Capillary tubes were filled with a solution of ricin in normal salt-solution of varying strength, 1 to 100,000, 1 to 200,000, 1 to 500,000, 1 to 1,000,000, and introduced beneath the skin of rabbits. Control-tubes of sterilized physiologic salt-solution were also introduced. After twenty hours the ricin-solution of 1 to 100,000 proved to be strongly chemotactic, the open end of the tube being plugged by a thrombus of leukocytes, fibrin, and granular material. The weaker solutions exhibited positive chemotactic properties directly in proportion to the concentration. Only a few leukocytes were found in the tubes containing simple salt-solution.

There remains one other class of ricin-cases, those of chronic character. These are more difficult to obtain, and very small doses must therefore be employed. I give a typical case.

On November 9th, a guinea-pig, weighing 600 grams, received 1.5 c.c. of a 1 to 200,000 solution of ricin, subcutaneously. There was considerable local reaction; a

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Aetiologie der acuten Eiterungen, Leipzig, 1889. O. Hertwig: Ueber die physiologische Grundlage der Tuberculin-wirkung, Jena, 1891.

hard node appeared over the site of injection, which, however, gradually disappeared. The animal lost weight for several days, but ten days subsequently to the inoculation had about regained the loss. On December 11th, seeming to have recovered, it was allowed to run about freely, and was often noticed to eat voraciously. Notwithstanding this fact, it grew more and more emaciated until December 29th, when its death occurred. At this time its weight was 270 grams.

At the autopsy the emaciation was extreme. The lymphatic glands were not enlarged; the spleen was small and dark, the liver dark. The intestines contained semi-fluid, mucus-like and turbid contents, and the mucous membrane was much attenuated. Cultures made from the organs were entirely negative.

This picture is strikingly different from that observed in the other class of cases, and presents the features of a profound disturbance of nutrition. The microscopic examination of the tissues showed by the amount of iron-containing pigment in the spleen and liver that great hemolysis had taken place. But of especial interest is the occurrence of atrophic patches in the kidneys.

Up to the present time we have considered the histologic lesions that are produced by the vegetable toxic proteids. I would now ask attention to those caused by toxic agents of animal origin.

Thus far my studies have been confined to the action of the blood-serum of man and dogs upon rabbits. We know from the experience of Landois,[1] Ponfick,[2] Daremberg,[3] Buchner,[4] Carter,[5] and others, that the immediate effect of the injection of serum is the destruc- of the red and colorless blood-corpuscles. The animals,

[1] Landois: Med. Centralblatt, 1874, p. 419.

[2] Ponfick: Berl. klin. Wochenschrift, 1874, No. 28. Ponfick u. Bamberg: Arch. f. path. Anat., Bd. lxii, p. 273.

[3] Daremberg: Soc. de Biologie, 1891, p. 719.

[4] Buchner: Münchener med. Wochenschrift, 1892, p. 120.

[5] Carter: University Med. Magazine, 1893, p. 170.

as often happens, may succumb immediately, in consequence of thrombosis of the right side of the heart and pulmonary artery, the respiratory function ceasing before the heart's action. Not uncommonly the animals experience less severe effects, and after a variable period of depression, increased frequency of respiration, weakness and hemoglobinuria, apparently recover.

I have found dog's serum strongly globulicidal for rabbits. This statement is based upon the common occurrence of hemoglobinuria that lasted from one to two days, as well as upon actual counts of the number of red blood-corpuscles. Doses of 1.5 per cent. of the body-weight were usually fatal to rabbits. In these cases death occurred either immediately or after ten or twelve hours. Quantities of one per cent. of the body-weight caused profound disturbances, including hemoglobinuria and albuminuria, and less commonly anuria, and in a few instances immediate death. Some of these animals survived several days or weeks, and some even appeared to recover. They were, however, as we have seen, more susceptible to subsequent inoculations.

When death follows at once upon, or soon after the inoculations, it is usual to find thrombi in the right side of the heart, which may extend into the pulmonary artery and its main branches. The rapid breaking up of the corpuscles liberates the fibrin-ferment, with consecutive coagulation of the blood in the vessels. The experiments of Naunyn[1] have shown that the red corpuscles yield the necessary ferment, and those of Schmidt and others that the white corpuscles, which are likewise destroyed by the foreign serum, are especially rich in this substance.

There are cases, however, in which great destruction of corpuscles has occurred, but in which coagulation has not ensued, and, indeed, under these circumstances the coagulability of the blood is diminished. We owe

[1] Archiv für exp. Pathologie, Bd. i.

to the researches of Pekelharing,[1] Wooldridge,[2] Löwit,[3] Schmidt,[4] and others[5] a partial understanding of this difference. It will not be possible for me to enter into the details of this matter here; it will suffice to state that much will depend upon the capacity of the organism to dispose of the liberated fibrin-factors and upon their behavior with regard to the calcium-salts of the plasma.

The cases already alluded to, in which the death of the animal is delayed for a time, are of especial interest, as they show certain well-marked lesions. While the observations that I have made are more complete as regards the action of the serum of the dog upon rabbits, the serum derived from human beings apparently acts in much the same way. Permit me to say that these lesions resemble, but are not identical with, those described in connection with the toxalbumins of diphtheria, ricin, and abrin.

The tissues of animals dead of serum-poisoning which I have so far studied consist of the spleen, liver, kidneys, and lymph-glands. In the acute cases the spleen shows a tolerably rich fragmentation of nuclei situated especially in the Malpighian bodies; the liver contains foci of necrosis of liver-cells, and the renal epithelium is degenerated and many methemoglobin-casts block the kidney-tubules.

Of especial interest is an animal that died on the thirteenth day in which very extensive lesions were found:

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[1] Pekelharing: Festschrift f. Virchow, 1891, Untersuchungen über das Fibrin-ferment, Amsterdam, 1892; Ueber die Gerinnung des Blutes, Deutsche med Wochenschrift, 1892.

[2] Wooldridge: Die Gerinnung des Blutes. Leipzig, 1891.

[3] Löwit: Studien zur Physiologie und Pathologie des Blutes und der Lymph. Jena, 1892.

[4] A. Schmidt: Zur Blutlehre, Leipzig, 1892.

[5] Artur et Pagès, Arch. de physiol. ii, 1890. Lilienfeld, Du Bois-Reymond's Archiv, 1892, pp. 115, 167, and 500.

A rabbit received on December 25, 1893, 1 per cent. of dog's serum, twenty-four hours old, intra-venously, and was dead in twenty minutes after completion of the inoculation. A second rabbit weighing 1200 grams received ½ of 1 per cent. of serum from the same source. There were no immediate perceptible effects. After the lapse of thirty minutes the expressed urine contained hemoglobin-casts. For some days following the inoculation the animal appeared to be in perfect health, but about the seventh day it began to lose in weight, grew perceptibly weaker from day to day, and died on January 6, 1894.

The autopsy showed great emaciation. The peritoneum contained an excess of fluid; the axillary lymph-glands were enlarged; the intestinal lymphatic apparatus was apparently normal. The liver, which was firm in consistence, was roughened externally and dark in color. The kidneys appeared small; their capsules were not adherent, but their surface was granular. In the spleen, which was enlarged, were a number of white wedge-shaped areas, the bases at the capsule, and quite firm in consistence. Attached to the chordæ tendineæ of the tricuspid valve was a calcified thrombus as large as a split-pea. The urine contained albumin and tube-casts.

The microscopic changes were those of chronic interstitial processes in the liver and kidney. In the latter the tubules were in places atrophied and surrounded by a new growth of connective tissue; in other places the tubules were dilated and the epithelium lining then degenerated; but it was in the liver that the pathologic alterations could best be studied. The chronic changes were well-marked here also, and were such an accurate reproduction of cirrhosis in human beings that a separate description seems superfluous. Areas of newly formed and forming connective tissue, proceeding from the portal spaces and also from the capsule, were irregularly distributed throughout the organ; newly formed bile-ducts were numerous; but what was of especial moment was the association with this of another pro-

cess, viz., acute degenerative changes in the liver-substance, which were often distinctly the starting-places of the sclerotic process.

In rabbits that died at a somewhat earlier period, after five or six days, I have not infrequently found coagulation-necrosis of liver-cells. To these areas leukocytes are not attracted in such numbers as in cases of diphtheria or poisoning with abrin or ricin; and in keeping with this difference I have found that capillary tubes containing dog's serum, when introduced beneath the skin of rabbits and removed after twenty-six hours, showed very slight positive chemotaxis only.

It is possible in the chronic cases to follow the progress of the pathologic process. The degenerative changes and the new growth of connective tissue can be studied side by side. There is necrosis and disintegration of liver-cells, emigration of leukocytes and growth of connective tissue. The new tissue comes both from the surface-capsule and from the inter-lobular connective tissue, but not exclusively from these. Independent new-formations in the midst of the lobule occur, just as there are independent foci of necrosis in this situation. Here and there, where the connective tissue is advancing, the fast disappearing remains of necrotic cells can be seen; but where the sclerotic tissue is already well developed and new bile-ducts are well formed, this is not always possible. Finally, in the spleen, the white nodules resembling infarcts are masses of fibrous tissue.

The changes found in the organs in cases of serum-poisoning are, if I mistake not, of no slight importance. They indicate that it is necessary to enlarge our views of the damage that serum is capable of doing, a damage not limited to the corpuscular elements of the blood, for the tissue-cells are not indifferent to its action.

In the end the differences in the action upon the blood and tissues of the toxic agents discussed are not so great as their correspondences—a fact that emphasizes their

chemic similarity. These substances so affect the tissue-elements as to cause degeneration and death, and their effects are followed by reparative processes that do not restore the integrity of the tissues. The relation that Weigert[1] several years ago maintained between parenchymatous degeneration and interstitial tissue-proliferation in the kidney, and which Ackermann[2] urged for the liver in chronic phosphorus-poisoning in rabbits, I have been able to follow in the cases mentioned in both organs, although most satisfactory in the liver.

Moreover, the chronic lesions, such as we have just studied, seem to be open to us for experimental study. Heretofore we have had to content ourselves with the production of degenerative changes, and have been excluded from imitating by experiments on lower animals the chronic proliferative processes in the internal organs of man.

In conclusion, the study of the tissue-changes that I have had the privilege of bringing before you has brought us from the consideration of acute degenerative changes of a focal character due to soluble poisons to the realization of the probability of their playing an important part in the production of chronic interstitial changes in animals, which find in cirrhosis of the liver and chronic nephritis analogies in human beings.

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[1] Volkmann's Sammlung klin. Vorträge, 1879, Nos. 162, 163.

[2] Virchow's Archiv, 1889, Bd. 115.